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(54) Title: ANTISENSE NUCLEIC ACID FOR THE TREATMENT OF DISEASES IN WHICH EXPRESSION OF bFGF, PDGF-A OR PDGF-B PLAYS A PATHOGENIC ROLE

(57) Abstract

A compound which is capable of preventing and treating neoplastic diseases and/or autoimmune diseases in which expression of PDGF-A, PDGF-B and/or bFGF plays a pathogenic role.

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ANTISENSE NUCLEIC ACID FOR THE TREATMENT OF DISEASES IN WHICH EXPRESSION OF bFGF, PDGF-A OR PDGF-B PLAYS A PATHOGENIC ROLE.

The present invention is related to an antisense-nucleic acid or effective derivatives thereof hybridizing with an area of the messenger RNA (mRNA) or the DNA, encoding platelet derived growth factor-A (PDGF-A) or platelet derived growth factor-B (PDGF-B) or basic fibroblast growth factor (bFGF), a pharmaceutical composition, comprising an antisense nucleic acid or effective derivatives thereof hybridizing with an area of the messenger RNA (mRNA) or the DNA, encoding PDGF-A or PDGF-B or bFGF as well as the use of said antisense nucleic acids and derivatives thereof for the manufacturing of a pharmacautieal composition for the treatment of neoplasms, for the inhibtion of pathological angiogenesis or the treatment of rheumatoid arthritis and other autoimmune diseases.

PDGF-A and PDGF-B as well as bFGF are growth factors, secreted by a variety of neoplastic cells including glioma cells, pancreas carcinoma cells, osteosarcoma cells, AIDS-related Kaposi's sarcoma cells, gastric carcinoma cells, lung carcinoma cells and melanoma cells and stimulate neoplastic cell growth in an autocrine manner. All three growth factors also stimulates neoangiogenesis in solid tumors. Furthermore,

these factors may promote angiogenesis and pathological formation of tissue deposits in autoimmune diseases.

It is an object of the present invention to provide a compound for the treatment of neoplasms, autoimmune diseases and diseases involving pathological angioneogenesis. Surprisingly the antisense nucleic acids described below, could strongly inhibit tumor cell growth, capillary endothelial cell growth and fibroblast cell growth even when the purified growth factors were added to the culture. This suggests that these growth factors exert growth regulatory effects by mechanisms independent of exogenous binding of these factors to their known receptors on the cell surface. Thus, the intracellularly produced polypetides or fragments thereof may bind regulatory sites on the cellular DNA or to protein or to intra cellular receptor sites. Alternatively, the oligonucleotides may act as aptamers.

According to the invention antisense nucleic acids or effective derivatives thereof which hybridize with an area of the mRNA or DNA coding for PDGF-A or PDGF-B or bFGF can effectively treat the diseases addressed above. The antisense nucleic acid is able to hybridize with regions of the PDGF-A or PDGF-B or bFGF mRNAs. It is understood by the skilled person that fragments of the antisense nucleic acids and antisense nucleic acids containing these sequences work according to the invention so long as production of the PDGF-A or PDGF-B or bFGF polypeptides is reduced or inhibited.

According to the invention the antisense-oligonucleotides are obtainable by solid phase synthesis using phosphite triester chemistry by growing the nucleotide chain in 3'-5' direction in that the respective nucleotide is coupled to the first nucleotide which is covalently attached to the solid phase comprising the steps of

- cleaving 5'DMT protecting group of the previous nucleotide,

- adding the respective nucleotide for chain propagation,

- modifying the phosphite group subsequently cap unreacted 5'-hydroxyl groups and

- cleaving the oligonucleotide from the solid support,

- followed by working up the synthesis product.

The chemical structures of oligodeoxy-ribonucleotides are given in figure 1 as well as the respective structures of antisense oligo-ribonucleotides are given in figure 2. The oligonucleotide chain is to be understood as a detail out of a longer nucleotide chain.

In figure 1 lit. B means an organic base such as adenine (A), guanine (G), cytosine (C) and thymine (T) which are coupled via N9(A,G) or N1(D,T) to the desoxyribose. The sequence of the bases is the reverse complement of the genetic target sequence (mRNA-sequence). The modifications used are

1. Oligodeoxy-ribonucleotides where all R^1 are substituted by

1.1 $R^1 = O$
1.2 $R^1 = S$
1.3 $R^1 = F$
1.4 $R^1 = CH_3$
1.5 $R^1 = OEt$

2. Oligodeoxy-ribonucleotides where R^1 is varied at the internucleotide phosphates within one oligonucleotide

5' B-p-B-p-B-p-(B-p-)$_n$B-p-B-p-B-p-B 3'

$$\begin{matrix} | & | & | & | & | & | & | \\ R^{1a} & R^{1a} & R^{1a} & R^{1b} & R^{1a} & R^{1a} & R^{1a} \end{matrix}$$

where B = deoxy-ribonucleotide dA, dC, dG or dT depending on gene sequence
p = internucleotide phosphate
n = an oligodeoxy-ribonucleotide stretch of length 6 - 20 bases

2.1	R^{1a} = S;	R^{1b} = O
2.2	R^{1a} = CH_3;	R^{1b} = O
2.3	R^{1a} = S;	R^{1b} = CH_3
2.4	R^{1a} = CH_3;	R^{1b} = S

3. Oligodeoxy-ribonucleotides where R^1 is alternated at the internucleotide phosphates within one oligonucleotide

$$5' \quad B\text{-}p\text{-}(B\text{-}p\text{-}B\text{-}p)_n\text{-}B\text{-}p\text{-}B \quad 3'$$

$$\begin{matrix} | & | & | & | \\ R^{1a} & R^{1b} & R^{1a} & R^{1b} \end{matrix}$$

where B = deoxy-ribonucleotide dA, dC, dG or dT depending on gene sequence
p = internucleotide phosphate
n = an oligodeoxy-ribodinucleotide stretch of length 4 - 12 dinucleotides

3.1	R^{1a} = S;	R^{1b} = O
3.2	R^{1a} = CH_3;	R^{1b} = O
3.3	R^{1a} = S;	R^{1b} = CH_3

4. Any of the compounds 1.1 - 1.5; 2.1 - 2.4; 3.1 - 3.3 coupled at R^2 with the following compounds which are covalently coupled to increased cellular uptake

4.1 cholesterol
4.2 poly(L)lysine
4.3 transferrin

5. Any of the compounds 1.1 - 1.5; 2.1 - 2.4; 3.1 - 3.3 coupled at R^3 with the following compounds which are covalently coupled to increase cellular uptake

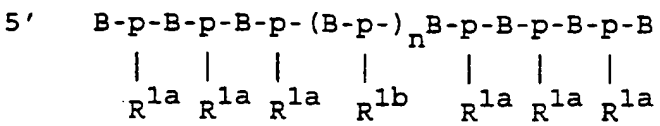
5.1 cholesterol
5.2 poly(L)lysine
5.3 transferrin

In the case of the RNA-oligonucleotides (figure 2) are the basis (adenine (A), guanine (G), cytosine (C), uracil (U)) coupled via N9 (A,G) or N1 (C,U) to the ribose. The sequence of the basis is the reverse complement of the genetic target sequence (mRNA-sequence). The modifications in the oligonucleotide sequence used are as follows

6. Oligo-ribonucleotides where all R^1 are substituted by

6.1 $R^1 = O$
6.2 $R^1 = S$
6.3 $R^1 = F$
6.4 $R^1 = CH_3$
6.5 $R^1 = OEt$

7. Oligo-ribonucleotides where R^1 is varied at the internucleotide phosphates within one oligonucleotide

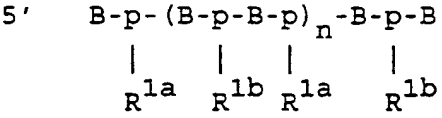


where B = ribonucleotide A, C, G or T depending

on gene sequence
p = internucleotide phosphate
n = an oligo-ribonucleotide stretch of length 4 - 20 bases

7.1 R^{1a} = S; R^{1b} = O
7.2 R^{1a} = CH_3; R^{1b} = O
7.3 R^{1a} = S; R^{1b} = CH_3
7.4 R^{1a} = CH_3; R^{1b} = S

8. Oligo-ribonucleotides where R^1 is alternated at the internucleotide phosphates within one oligonucleotide



where B = ribonucleotide A, C, G or T depending on gene sequence
p = internucleotide phosphate
n = an oligo-ribodinucleotide stretch of length 4 - 12 dinucleotides

8.2 R^{1a} = S; R^{1b} = O
8.2 R^{1a} = CH_3; R^{1b} = O
8.3 R^{1a} = S; R^{1b} = CH_3

9. Any of the compounds 6.1 - 6.5; 7.1 - 7.4; 8.1 - 8.3 coupled at R^2 with the following compounds which are covalently coupled to increase cellular uptake

9.1 cholesterol
9.2 poly(L)lysine
9.3 transferrin

10. Any of the compounds 6.1 - 6.5; 7.1 - 7.4; 8.1 - 8.3 coupled at R^3 the following compounds are covalently

coupled to increased cellular uptake

10.1 cholesterol
10.2 poly(L)lysine
10.3 transferrin

11. Any of the compounds 6.1 - 6.5; 7.1 - 7.4; 8.1 - 8.3; 9.1 - 9.3; 10.1 - 10.3 where all R^4 are substituted by

11.1 $R^4 = O$
11.2 $R^4 = F$
11.3 $R^4 = CH_3$

In a preferred embodiment the PDGF-A antisense-oligonucleotide has the sequence as disclosed in the sequence listing under Seq. ID No. 1 - 6, having a DNA- or RNA-type structure.

In a preferred embodiment the PDGF-B antisense-oligonuclotide has the sequence as disclosed in the sequence listing under Seq. ID No. 7 - 12, having a DNA- or RNA-type structure.

In a preferred embodiment the bFGF antisense-oligonucleotide has the sequence as disclosed in the sequence listing under Seq. ID No. 13 - 27, having a DNA- or RNA-type structure.

In a preferred embodiment of these oligonucleotides they are phosphorothioate derivatives, having a DNA- or RNA-type structure.

It is possible that one single individual sequence as mentioned above works as an antisense nucleic acid or oligonucleotide structure according to the invention. However, it is also possible that one strand of nucleotides comprises more than one of the sequences as mentioned above directly covalently linked or with other nucleotides covalently linked in between. Preferably, individual oligonucleotides are

addressed.

The randomized control sequence is disclosed in the sequence listing under Seq. ID No. 28.

In a preferred embodiment of these oligo-nucleotides they are phosphorothioate derivatives.

Modifications of the antisense-oligonucleotides are advantageous since they are not as fast destroyed by endogeneous factors when applied as this is valid for naturally occuring nucleotide sequences. However, it is understood by the skilled person that also naturally occuring nucleotides having the disclosed sequence can be used according to the invention. In a very preferred embodiment the modification is a phosphorothioate modification.

The synthesis of the oligodeoxy-nucleotide of the invention is described as an example in a greater detail as follows.

Oligodeoxy-nucleotides were synthesized by stepwise 5'-addition of protected nucleosides using phosphite triester chemistry. The nucleotide A was introduced as 5'dimethoxytrityl-deoxyadenosine(N-benzoyl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite (0.1 M); C was introduced by a 5'-dimethoxytrityl-deoxycytidine(N^4-benzoyl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite; G was introduced as 5'-dimethoxytrityl-deoxyguanosine(N^8-isobutyryl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite and the T was introduced as 5'-dimethodytrityl-deoxythymidine-N,N'-diisopropyl-2-cyanoethyl phosphoramidite. The nucleosides were preferably applied in 0.1 M concentration dissolved in acetonitrile.

Synthesis was performed on controlled pore glass particles of approximately 150 µm diameter (pore diameter 500 Å) to which the most 3' nucleoside is covalently attached via a long-chain alkylamin linker (average loading 30 µmol/g solid

support).

The solid support was loaded into a cylindrical synthesis column, capped on both ends with filters which permit adequate flow of reagents but hold back the solid synthesis support. Reagents were delivered and withdrawn from the synthesis column using positive pressure of inert gas. The nucleotides were added to the growing oligonucleotide chain in 3'-> 5' direction. Each nucleotide was coupled using one round of the following synthesis cycle:

Cleave 5'DMT (dimethoxytrityl) protecting group of the previous nucleotide with 3-chloroacetic acid in dichloromethane followed by washing the column with anhydrous acetonitrile. Then simultaneously one of the bases in form of their protected derivative depending on the sequence was added plus tetrazole in acetonitrile. After reaction the reaction mixture has been withdrawn and the phosphite was oxidized with a mixture of sulfur (S_8) in carbon disulfid/pyridine/-triethylamine. After the oxidation reaction the mixture was withdrawn and the column was washed with acetonitrile. The unreacted 5'-hydroxyl groups were capped with simultaneous addition of 1-methylimidazole and acetic anhydryide/lutidine-/tetrahydrofuran. Thereafter, the synthesis column was washed with acetonitrile and the next cycle was started.

The work up procedure and purification of the synthesis products occured as follows.

After the addition of the last nucleotide the deoxynucleotides were cleaved from the solid support by incubation in ammonia solution. Exocyclic base protecting groups were removed by further incubation in ammonia. Then the ammonia was evaporated under vacuum. Full-length synthesis products still bearing the 5'DMT protecting group were separated from shorter failure contaminants using reverse phase high performance liquid chromatography on silica C_{18} stationary

phase. Eluents from the product peak were collected, dried under vacuum and the 5'-DMT protecting group cleaved by incubation in acetic acid which was evaporated thereafter under vacuum. The synthesis products were solubilized in the deionized water and extracted three times with diethylether. Then the products were dried in vacuo. Another HPLC-AX chromatography was performed and the eluents from the product peak were dialysed against excess of Trisbuffer as well as a second dialysis against deionized water. The final products were lyophilized and stored dry.

The anisense-nucleic acid of the invention can be used as pharmaceutical composition or medicament. This medicament can be used for treating neoplasms in which the expression of platelet derived growth factor or basic fibroblast growth factor is of relevance for the pathogenicity. It can be used to reduce neoplastic cell growth in cells expressing these growth factors, and to inhibit pathological angiogenesis. Furthermore, it can be used to treat rheumatoid arthritis and other autoimmune diseases in which the production of PDGF-A, PDGF-B or bFGF is of pathogenetic relevance.

The effect of PDGF-A and PDGF-B and bFGF specific antisense oligonucleotides on neoplastic cell growth was investigated. It was demonstrated that antisense oligodeoxynucleotides as well as phosphorothioate modified nucleic acids, complementary to PDGF and bFGF mRNAs could specifically inhibit PDGF protein expression and bFGF protein expression respectively. Furthermore, they reduced cell proliferation in mammary carcinoma, glioma, pancreas carcinoma and melanoma cells with surprising efficiency.

Surprisingly, cell extracts from tumor cells treated with the specific antisense oligodeoxynucleotides targeted against either of the three growth factors did not induce angioneogenesis, while extracts from untreated cells were highly angiogenic.

The invention is further explained by the following non limiting example.

Example

Enzyme-linked immunosorbent assay (ELISA)

Cell lysates were diluted in 50 mM carbonate buffer at pH 9.0 and immobilized on immunon II plates (Dynatech Laboratories, Inc.) overnight. Antigen solution was removed and 200 µl/well phosphate buffered saline (PBS)/ 1%/BSA/0.02% azide were added to block non-specific protein binding. Following incubation at room temperature for 2 h solution was removed. After washing with PBS plates were air dried for 3 h. Specific antibodies for PDGF-A, PDGF-B or bFGF (Oncogene or Santa Cruz Biotechnology Inc.) were added at 50 µl/well, diluted in blocking buffer. Following 1 h incubation at room temperature samples were removed and subsequently wells were washed four times with PBS/0.05% Tween 20. Then 50 µl of secondary antibody-phosphatase conjugate were added and removed after 1 h. Wells were washed with diethanolamine buffer (10 mM diethanolamine, 0.5 mM $MgCl_2$, pH 9,5). One tablet of Sigma 104 phosphatase substrate was dissolved in 5 ml diethanolamine buffer. 50 µl of the substrate solution were added per well. The reaction was stopped with 50 µl 0,1 M EDTA (pH 7.5) and plates were read on a microtitration plate reader.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Biognostik Gesellschaft fuer biomolekulare Diagnostik mbH
(B) STREET: Carl-Giesecke-Str. 3
(C) CITY: Goettingen
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 37079

(ii) TITLE OF INVENTION: Antisense nucleic acid for the treatment of neoplasms and auto-immune diseases and diseases involving pathological angioneogenesis, in which expression of the growth factors bFGF, PDGF-A or PDGF-B..

(iii) NUMBER OF SEQUENCES: 28

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCCAAGGTCC TCAT 14

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGAGTCTATC TCCA 14

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCAAAGAATC CTCACT 16

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CACATGCTTA GTGG 14

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCGTAAATG ACCG 14

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGGAATCTCG TAAATGAC 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGCAGCGAT TCAT 14

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGAGATCATC AAAGGA 16

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCAGCAATG GTCA 14

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GATCTCGAAC ACCT 14

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CACAATCTCG ATCTTTCT 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTTCTTAAA GATTGGCT 18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CACATACCAA CTGG 14

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGCTTGATGT GAGG 14

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAAGTTGTAG CTTGATGT 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTTGAAGTT GTAGCT 16

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGCTTGAAG TTGTAG 16

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GACACAACTC CTCT 14

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCCTTTGATA GACACAAC 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTCCTTTGAT AGACAC 16

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGTTAGCACA CACT 14

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGTAACGGTT AGCA 14

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGTAACACAT TTAGAAGC 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCATCCGTA ACAC 14

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCGGTAAGTA TTGTAGTT 18

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGTGTATTTC CTTGAC 16

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACATACCAAC TGGTGT 16

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTCCCTATAC GAAC 14

Claims

1. A compound which is capable of treating or preventing neoplasms and/or autoimmune diseases and/or diseases involving pathological angiogenesis, in which expression of PDGF-A, PDGF-B and/or bFGF plays a pathogenetic role.

2. The compound of claim 1 being an antisense nucleic acid or effective derivative thereof, said antisense nucleic acid hybridizing with an area of the messenger RNA (mRNA) and/or DNA encoding bFGF, PDGF-A and/or PDGF-B.

3. The compound of claims 1 and/or 2 wherein the PDGF-A antisense oligonucleotide comprises the following sequences identified in the sequence listing under Seq ID No. 1 - 6, having a DNA- or RNA-type structure.

   the PDGF-B antisense-oligonuclotide comprises the sequences identified in the sequence listing under Seq. ID No. 7 - 12, having a DNA- or RNA-type structure.

   and the bFGF antisense-oligonucleotide comprises the sequences identified in the sequence listing under Seq. ID No. 13 - 27, having a DNA- or RNA-type structure.

4. Antisense oligonucleotides of the claims 2 and 3 wherein the oligonucleotides are modified oligonucleotides such as phosphorothioate derivatives.

5. Antisense nucleic acid or -oligonucleotides according to any one of the claims 1 to 4 obtainable by solid phase synthesis using phosphite triester chemistry by growing the nucleotide chain in 3'-5' direction in that the respective nucleotide is coupled to the first nucleotide which is covalently attached to the solid phase comprising the steps of

- cleaving 5'DMT protecting group of the previous nucleotide,

- adding the respective nucleotide for chain propagation,

- modifying phosphite groups subsequently cap unreacted 5'-hydroxyl groups and

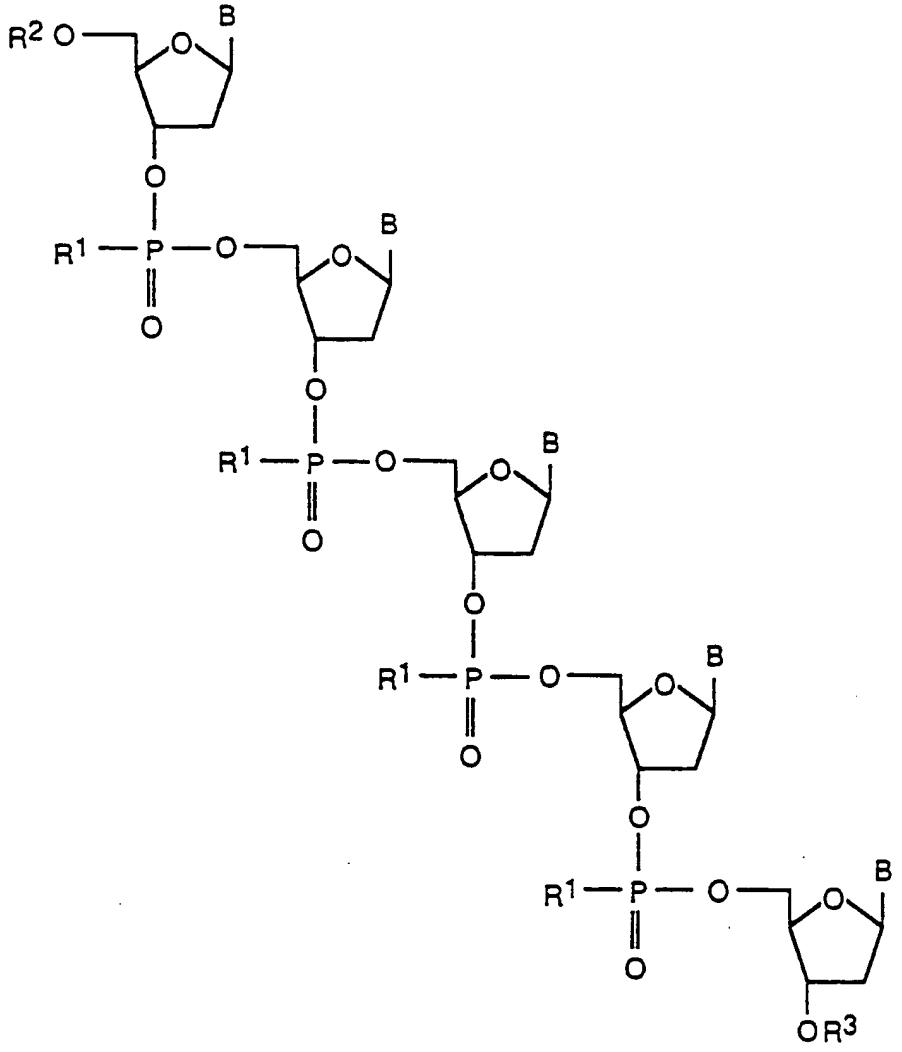
- cleaving the oligonucleotide from the solid support,

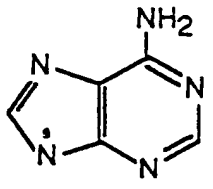
- followed by working up the synthesis product.

6. A pharmaceutical composition comprising an effective amount of a compound of any one of the claims 1 to 5 for the prevention and treatment of neoplasms and/or autoimmune diseases and/or diseases involving pathological angiogenesis, in which expression of PDGF-A, PDGF-B and/or bFGF plays a pathogenetic role.

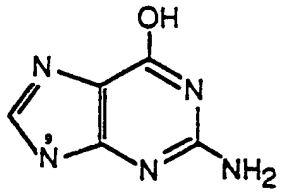
7. Use of a compound according to any one of the claims 1 to 5 for the preparation of a pharmaceutical composition for the treatment and or prevention of neoplasms and/or autoimmune diseases and/or diseases involving pathological angiogenesis related with the expression of PDGF-A, PDGF-B and/or bFGF.

8. Method of treating or preventing neoplasms and/or autoimmune diseases and/or diseases involving pathological angiogenesis by administering an effective amount of the compound according to any one of the claims 1 to 5 or a pharmaceutical composition of claim 6 to a patient suffering from disorders related with the expression of PDGF-A, PDGF-B and/or bFGF.

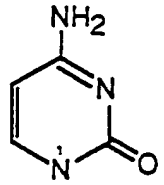




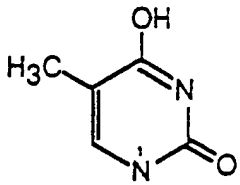
Adenine



Guanine

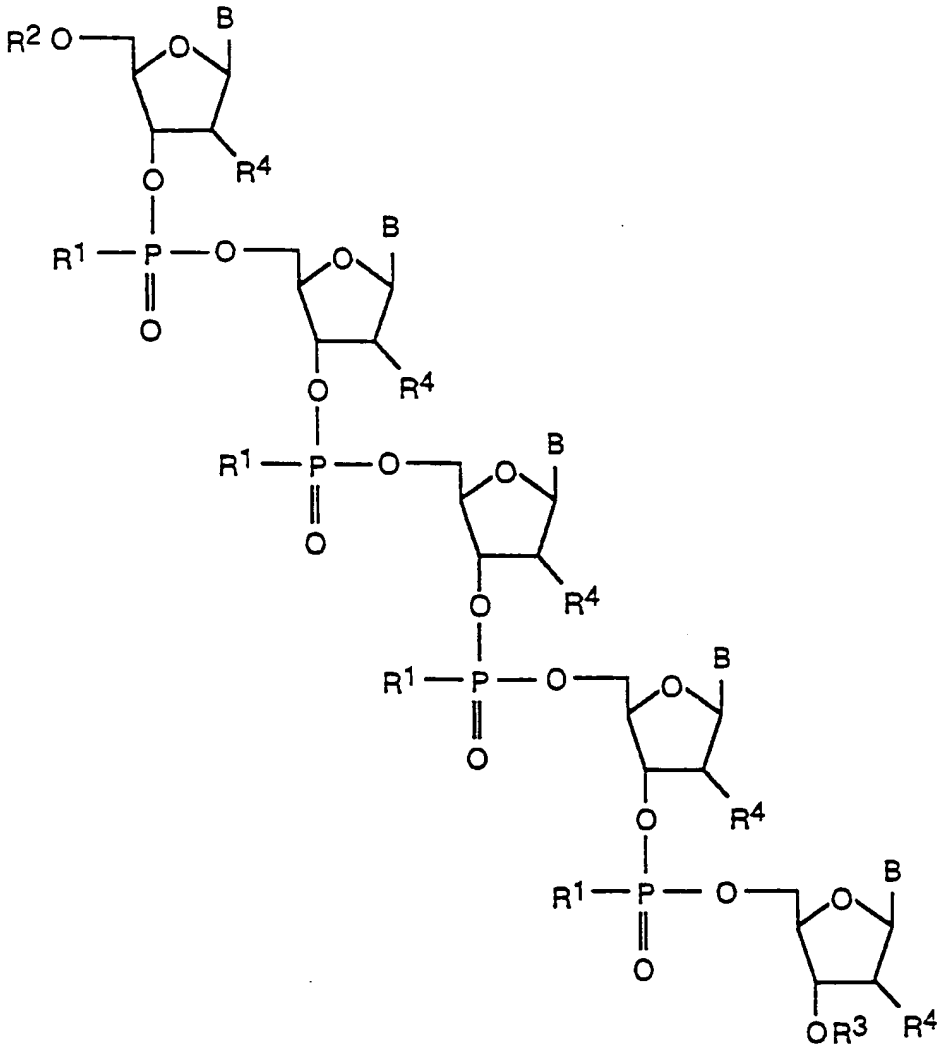


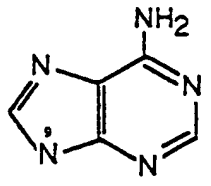
Cytosine



Thymine

Fig. 1





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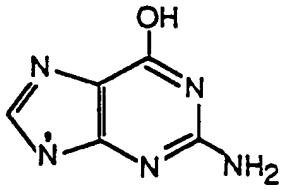
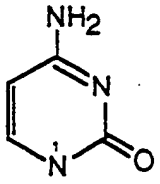
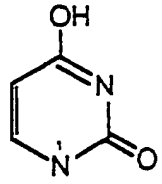


Guanine



Cytosine



Uracil

Fig. 2

INTERNATIONAL SEARCH REPORT

Inter. ıal Application No
PCT/EP 93/03461

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NEUROSURGERY, vol.33, no.4, October 1993 pages 679 - 684 BEHL, C. ET AL. 'Autocrine growth regulation in neuroectodermal tumors as detected with oligodeoxynucleotide antisense molecules' see the whole document	1-8
Y	---	3,5-7
Y	NATURE, vol.320, 24 April 1986, LONDON GB pages 695 - 699 BETSHOLZ, C. ET AL. 'cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumor cell lines' see figure 1	3
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 August 1994	21. 12. 94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	**Authorized officer** ANDRES S.M.

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol.193, no.2, 15 June 1993, DULUTH, MINNESOTA US pages 744 - 751 BEHL, C. ET AL. 'Autoinduction of platelet derived growth factor (PDGF) A-chain mRNA expression in a human melanoma cell line and growth inhibitory effects of PDGF A-chain mRNA-specific antisense molecules' see the whole document, and especially page 746, line 4	1-5,8
X	WO,A,92 17206 (THE VICTORIA UNIVERSITY OF MANCHESTER) 15 October 1992 see page 6, line 1 - line 4	1,2,6-8
Y	see page 11, line 10 - line 19 see claims 1,9	6,7
Y	ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, vol.22, no.6, June 1983, WEINHEIM DE pages 423 - 439 ECKSTEIN, F. 'Phosphorothioate analogues of nucleotides - tools for the investigation of biochemical processes' see page 426, chapter 2.3 see figure 12 see page 432, chapters 4.1 and 4.2	5
X	ERICKSON, R. & IZANT, J.:'Gene regulation: biology of antisense RNA and DNA'; 1992, RAVEN PRESS, Ltd., NEW YORK pages 317-328, cited in the application SCHLINGENSIEPEN, K.-H. & BRYSCH, W.: 'Phosphorothioate oligomers' see page 321, last paragraph - page 322, line 6	1,2,4,5, 8
X	HYPERTENSION, vol.16, no.3, September 1990 pages 325 - 326 ITOH, H. ET AL. 'Antisense oligonucleotides complementary to PDGF mRNA attenuate angiotensin II-induced vascular hypertrophy'	1,2,8
A	see abstract 46	6,7

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/EP 93/03461

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 8 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/ composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see annex

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-8 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1.- claims 1-8 (all partially): Antisense oligonucleotides hybridizing to PDGF-A mRNA or DNA, their use in the preparation of pharmaceutical compositions for the treatment of neoplasms, autoimmune diseases, or diseases involving pathological angiogenesis.

2.- claims 1-8 (all partially): Antisense oligonucleotides hybridizing to PDGF-B mRNA or DNA, their use in the preparation of pharmaceutical compositions for the treatment of neoplasms, autoimmune diseases, or diseases involving pathological angiogenesis.

3.- claims 1-8 (all partially): Antisense oligonucleotides hybridizing to bFGF mRNA or DNA, its use in the preparation of pharmaceutical compositions for the treatment of neoplasms, autoimmune diseases, or diseases involving pathological angiogenesis.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ..al Application No
PCT/EP 93/03461

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9217206	15-10-92	AU-A-	1436892	02-11-92
		EP-A-	0585242	09-03-94
		JP-T-	6506205	14-07-94